A systematic investigation of CID Q-TOF-MS/MS collision energies

A systematic investigation of CID Q-TOF-MS/MS collision energies to allow N- and O-glycopeptide identification by LC-MS/MS

Abstract

The MS investigation of synthetic glycopeptides revealed the optimum collision energies for identifying both the glycan and the peptide moiety of glycopeptides. Using this knowledge an acquisition method for the impact II Bruker QTOF was created. The usefulness of this method was proven by the application to a glycopeptide mixture.

Keywords:
glycopeptide,
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glycosylation site,
N-linked glycan,
O-linked glycan,
theoretical digest

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Introduction

Protein glycosylation is a dynamically regulated, non-template dependent process influenced by various factors such as age, sex, cell and tissue type as well as health status. This makes glycoproteins prime biomarker targets. In order to investigate the role individual glycoproteins play in the onset and progression of numerous diseases reliable and robust analytical approaches are crucial for determination of glycan composition in a protein/peptide specific manner. Mass spectrometry has developed as one of the most powerful tools for the sensitive identification and structure elucidation of glycopeptides. However, the optimal collision energy conditions required to obtain a maximum of information on both, the glycan as well as the peptide moiety have to date rarely been systematically investigated.

Methods

1. Glycopeptide synthesis

- Glycan building block extraction
- Solid phase glycopeptide synthesis
- C18 Purification

3. Evaluation + automated data analysis

- Immunoglobulins/ Fetuin
  - Nano-LC-MS/MS of tryptic digests
- Data analysis
  a) Classification of GP (incl. peptide moiety mass determination)
  b) Peptide-Protein matching (theoretical digest)
  c) Identification of glycan (GlycoQuest): compositions (putative structure suggestion)
  d) Combination of the results

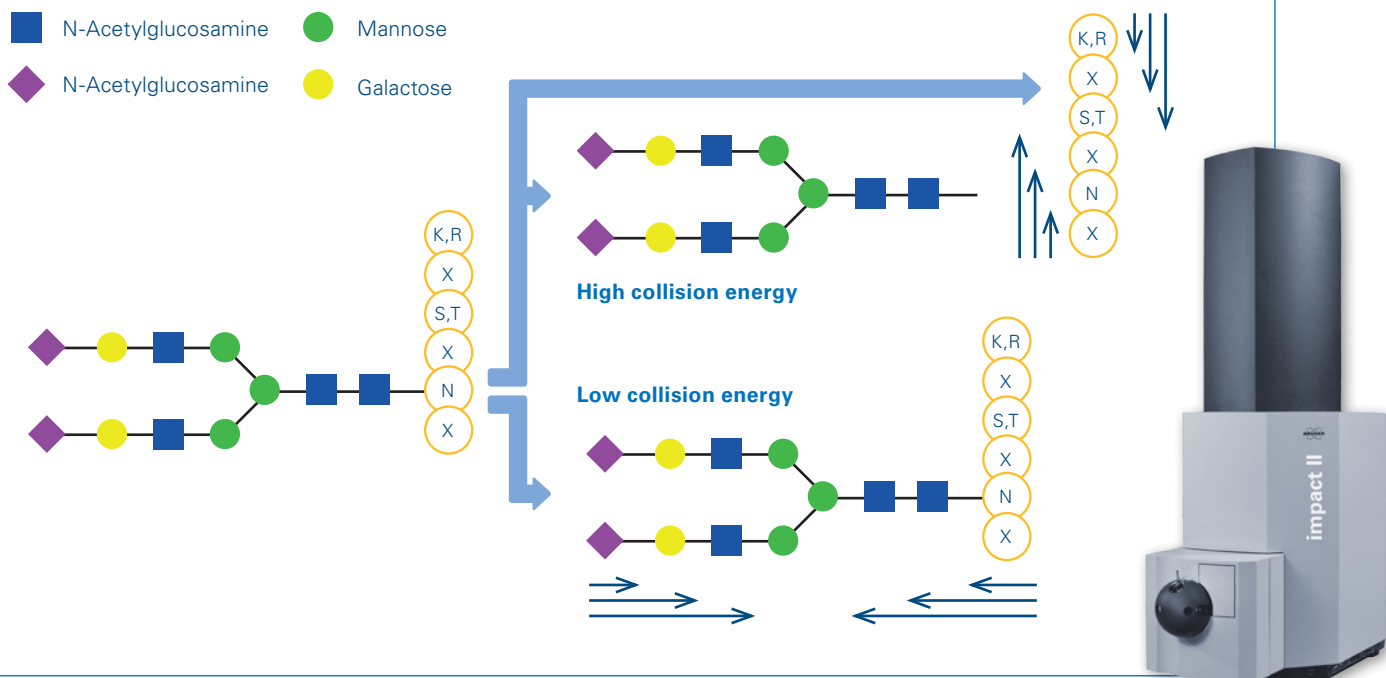### 2. Collision energy optimization Q-TOF (impact II)

Figure 1: Workflow. Glycopeptides were synthesized (1) and then used in Q-TOF instruments (impact II) for tuning collision energies for optimal glycan and peptide moiety fragmentation (2). Settings were applied on a set of glycoproteins and data automatically analyzed using ProteinScape 4.0 (3).

Results

Collision Energy Optima using synthetic glycopeptides

- Systematic investigation for four charge states (Fig. 2) provided clear collision energy optima for both, the peptide and the glycan part.
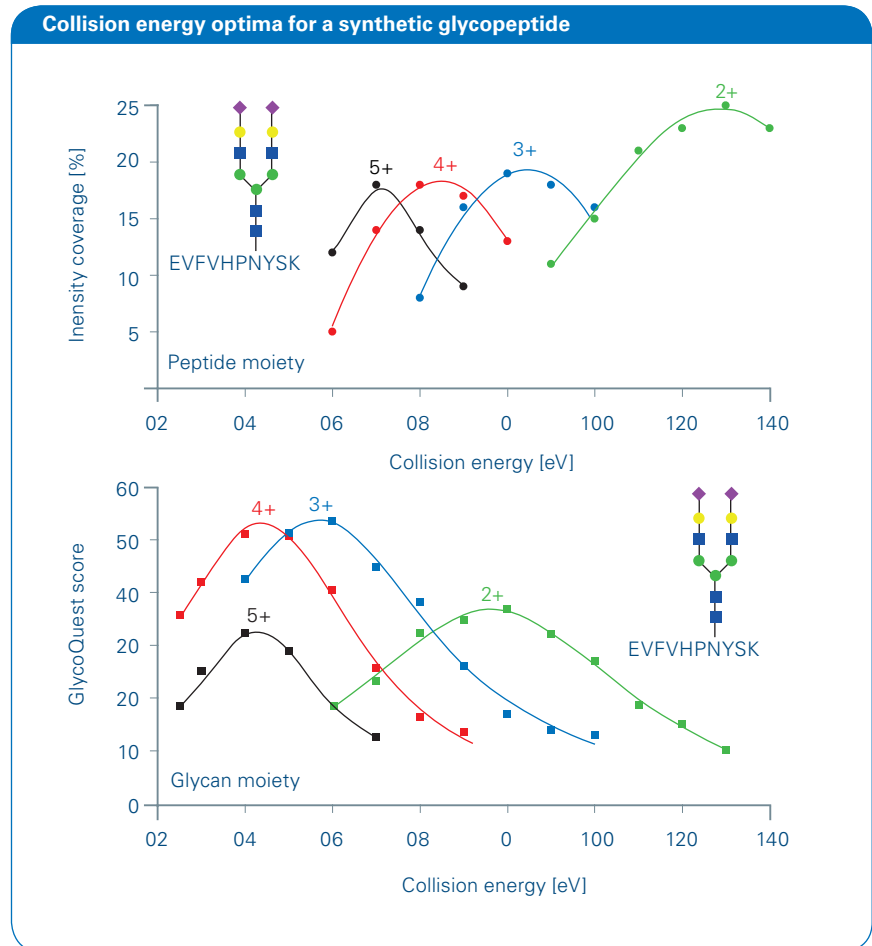- Optimum collision energies for glycan moieties are significantly lower than for the peptide portion.

Collision energy optima for a synthetic glycopeptide

Figure 2: Dependency of the quality criteria for the ID of the glycan and the peptide moiety of a synthetic glycopeptide from the applied collision energy.

Knowledge extension to a glycopeptide mixture glycopeptides

- Linear dependency of collision energy optimum and m/z ratio (Fig. 3)

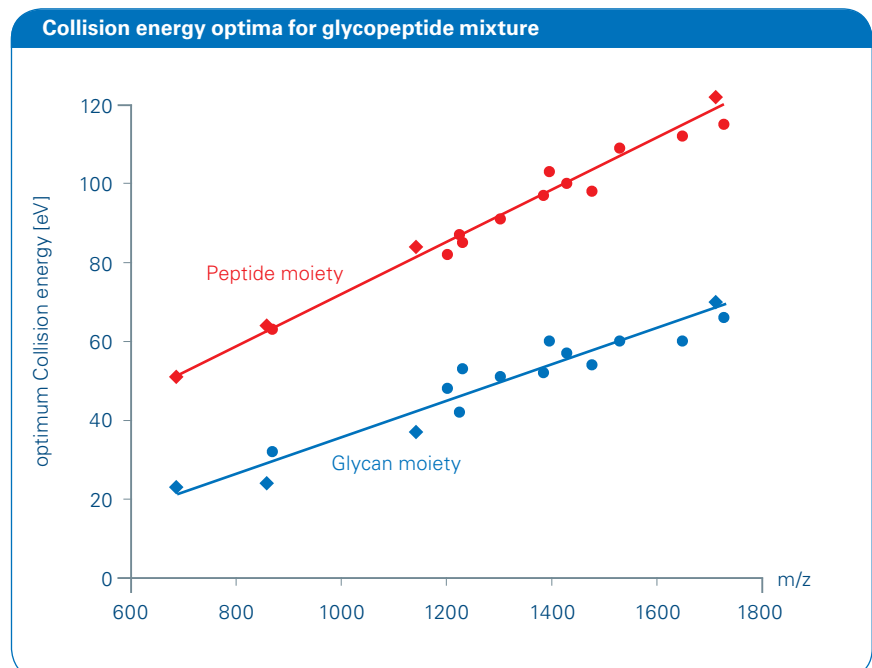**Collision energy optima for glycopeptide mixture**

Figure 3: Dependency of the optimal collision energy of the glycan and peptide portion from the m/z values.
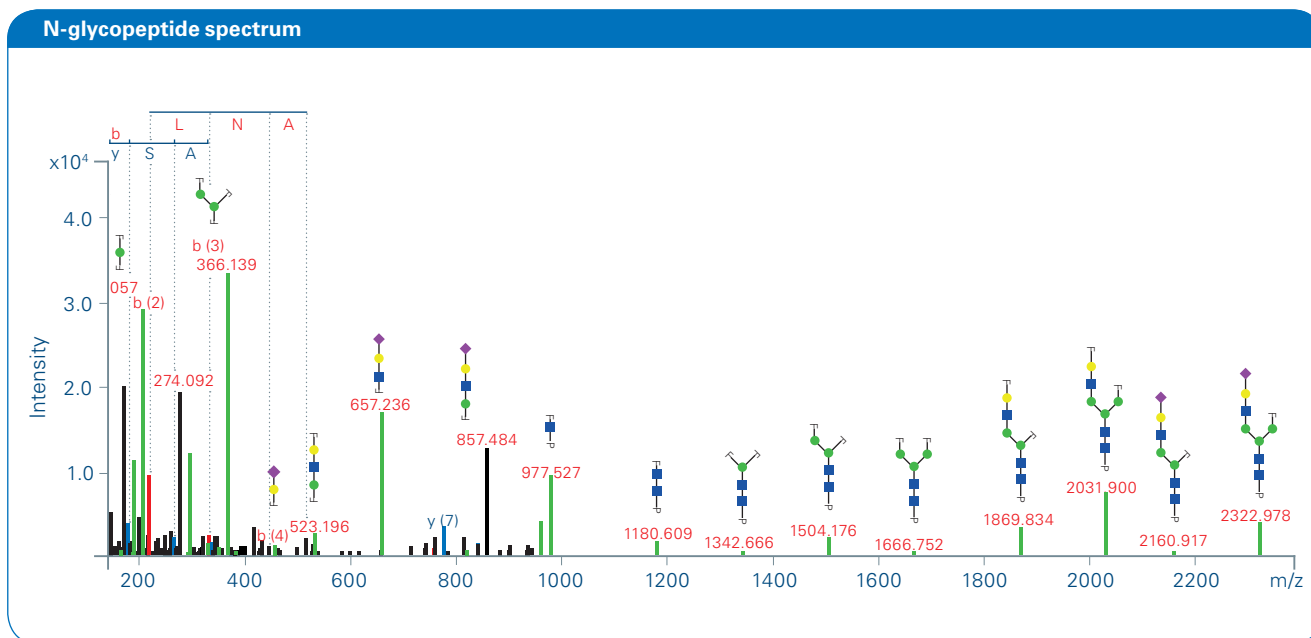
Figure 4: Representative spectrum of automatically assigned N- glycopeptides from IgD using the optimized Q-TOF collision energy stepping dissociation method for tryptic N-glycopeptide TLLNASR (N367) carrying a $Hex_5HexNAc_4NeuAc_2$ N-glycan.
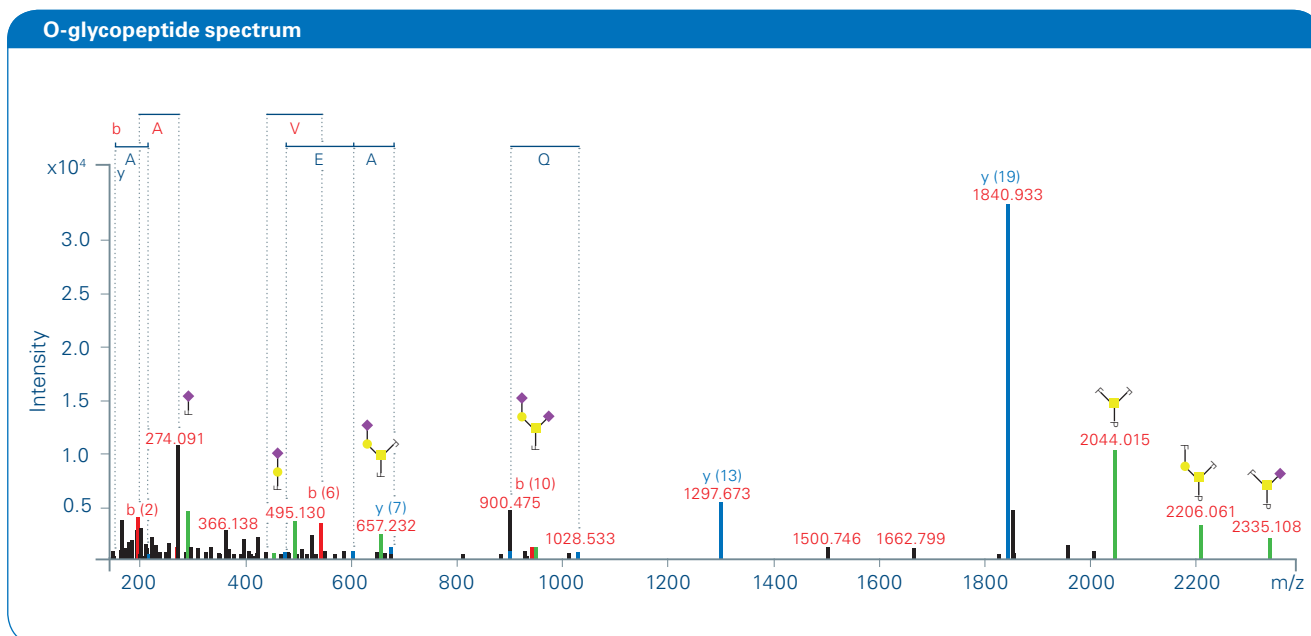

Figure 5: CID spectrum example for a tryptic IgD O-glycopeptide AQASSVPTAQPQAEGSLAK (Ser109, Ser110, Thr113) with $HexNAc_2NeuAc_2$ attached.

Application to tryptic digests from human Immunoglobulins

- Q-TOF CID spectra at the optimum collision energy for the peptide and the glycan moiety were summarized.
- This allowed the ProteinScape driven ID of the complete glycopeptides.

Identification of N-glycopeptides

Figure 4 shows one example of a CID spectrum where a glycan with composition $Hex_5HexNAc_4NeuAc_2$ is linked to a IgD peptide containing Asn 367. 25 further glycans were found linked to the same peptide sequence. They are listed in Table 1.

Identification of O-glycopeptides

The workflow described above is equally applicable on O-glycosylated peptides. An example for Hex_2NeuAc_2 linked to peptide AQASS VPTAQPQAEG SLAK is shown in Figure 5.

Table 1: Overview of identified N-glycan compositions linked to peptide R.TLLNASR.S of IgD.

Mr calc.	z	Peptide Score	Glycan Score	Glycan Composition
2421.0528	3	18.9	26.5	Hex3HexNAc5dHex1
2274.9949	3	11.4	23.5	Hex3HexNAc5
2671.1217	3	15.8	26.9	Hex4HexNAc4NeuAc1dHex1
2525.0638	3	5.1	19.0	Hex4HexNAc4NeuAc1
2583.1056	2	20.6	39.0	Hex4HexNAc5dHex1
2874.2010	2	18.4	30.5	Hex4HexNAc5NeuAc1dHex1
2728.1431	3	17.9	37.2	Hex4HexNAc5NeuAc1
2437.0477	3	20.1	46.4	Hex4HexNAc5
2542.0791	3	8.9	21.4	Hex5HexNAc4dHex1
2833.1745	3	17	28.9	Hex5HexNAc4NeuAc1dHex1
2687.1166	2	18.7	31.3	Hex5HexNAc4NeuAc1
3124.2699	2	18.6	37.3	Hex5HexNAc4NeuAc2dHex1
2978.2120	2	19.4	39.6	Hex5HexNAc4NeuAc2
2396.0212	3	13.1	30.2	Hex5HexNAc4
2745.1584	2	16.5	34.2	Hex5HexNAc5dHex1
3036.2539	3	17.3	26.1	Hex5HexNAc5NeuAc1dHex1
2890.1959	2	18.4	32.6	Hex5HexNAc5NeuAc1
3327.3493	2	18.8	32.6	Hex5HexNAc5NeuAc2dHex1
3181.2914	2	18.4	47.1	Hex5HexNAc5NeuAc2
2599.1005	3	17.9	45.9	Hex5HexNAc5
2907.2113	3	17.1	22.6	Hex6HexNAc5dHex1
3198.3067	3	15.6	25.4	Hex6HexNAc5NeuAc1dHex1
3052.2488	3	17.2	30.5	Hex6HexNAc5NeuAc1
3489.4021	4	14.1	25.4	Hex6HexNAc5NeuAc2dHex1
3343.3442	4	15.2	28.2	Hex6HexNAc5NeuAc2
2761.1534	3	14	37.7	Hex6HexNAc5

Conclusions

Complete glycopeptides can be characterized (Fig. 1) based on:

- Knowledge about the optimized collision energies for glycopeptide fragmentation

- Q-TOF MS technology for directed frag-mention of the glycan and the peptide moiety

- Bioinformatic platform for the identification of complete glycopeptides

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References

The art of destruction: http://link.springer.com/article/10.1007/s13361-015-1308-6